



95-0012 User Guide- TD-GC-Lonestar: How To Run a Sample Tube

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1 Introduction

The TD-GC-Lonestar system sold by Owlstone Medical Ltd gives the capability to analyse thermal desorption tubes using FAIMS on the Lonestar equipment.

The Gas Chromatograph (GC) system used is the Thermo Trace 1310 and the thermal desorption (TD) used is the Markes UNITY-xr.

This user guide explains the process for sample tube analysis, highlighting essential checks for correct TD-GC-LNS system function. The procedures for maintenance, quality control and data processing procedures, will be referred to, but are out of the scope of this document.

2 Definitions

Abbreviation/Term	Definition
OML	Owlstone Medical Ltd.
SOP	Standard Operation Procedure
TD	Thermal Desorption. The TD-GC-LNS system has the Markes UNITY-xr TD Unit integrated.
UNITY-xr	Thermal desorption platform with a sorbent tube oven integrated in where sample tubes can be loaded and desorbed for VOCs injection onto the GC column.
VOC	Volatile Organic Compound
GC	Gas Chromatography. The TD-GC-LNS system has the GC Thermo Trace 1310 integrated.
LNS	Lonestar System – its FAIMS chips enables to detect chemical species in gaseous state based on its characteristic ion mobility speed under an asymmetric electric field.
ATLAS	The name of the combined parts of the sampling unit supplied with Lonestar (Pneumatics Box, Heater Box, Split Flow Box, Sample Module)
MIC	Markes Instrument Control – TD software.
FAIMS	Field Asymmetric Ion Mobility Spectrometry
He	Helium, acts as carrier gas in the TD-GC-LNS system
N2	Nitrogen, acts as purge gas in the TD-GC-LNS system.

3 TD-GC-LNS system overview.

The breath samples are collected in tubes packed with sorbent particles, which able to bind selectively to VOC's of interest. Thermal desorption consists on inducing the analyte release from the sorbents by heating the sample tube. The analytes of interested are then directed onto the GC using a carrier gas flow.

The TD unit integrated in this system is the UNITY-xr. The breath samples are loaded onto the tube oven, which heats the tube and applies a backflush of clean Helium (carrier) gas. The He flow then drives the sample towards a focusing (cold) trap that splits and recollects the analytes. A second desorption step takes place on the cold trap, in where a backflush of He transports and introduces the re-collected sample into the GC column via a transfer line.

Analytes separation occurs as they elute along the column and a transfer line connecting the GC with the LNS. As the VOC's reach the LNS, its FAIMS sensor detects different compounds and generates data based on its differential ion mobility. The different instruments integrating the TD-GC-LNS are communicated in such a way that the cold trap desorption on the UNITY-xr triggers the GC method and data collection on the LNS.

Figure 1 shows a schematic view of the TD-GC-LNS with the main temperature and gas pressure ranges for correct system functioning.

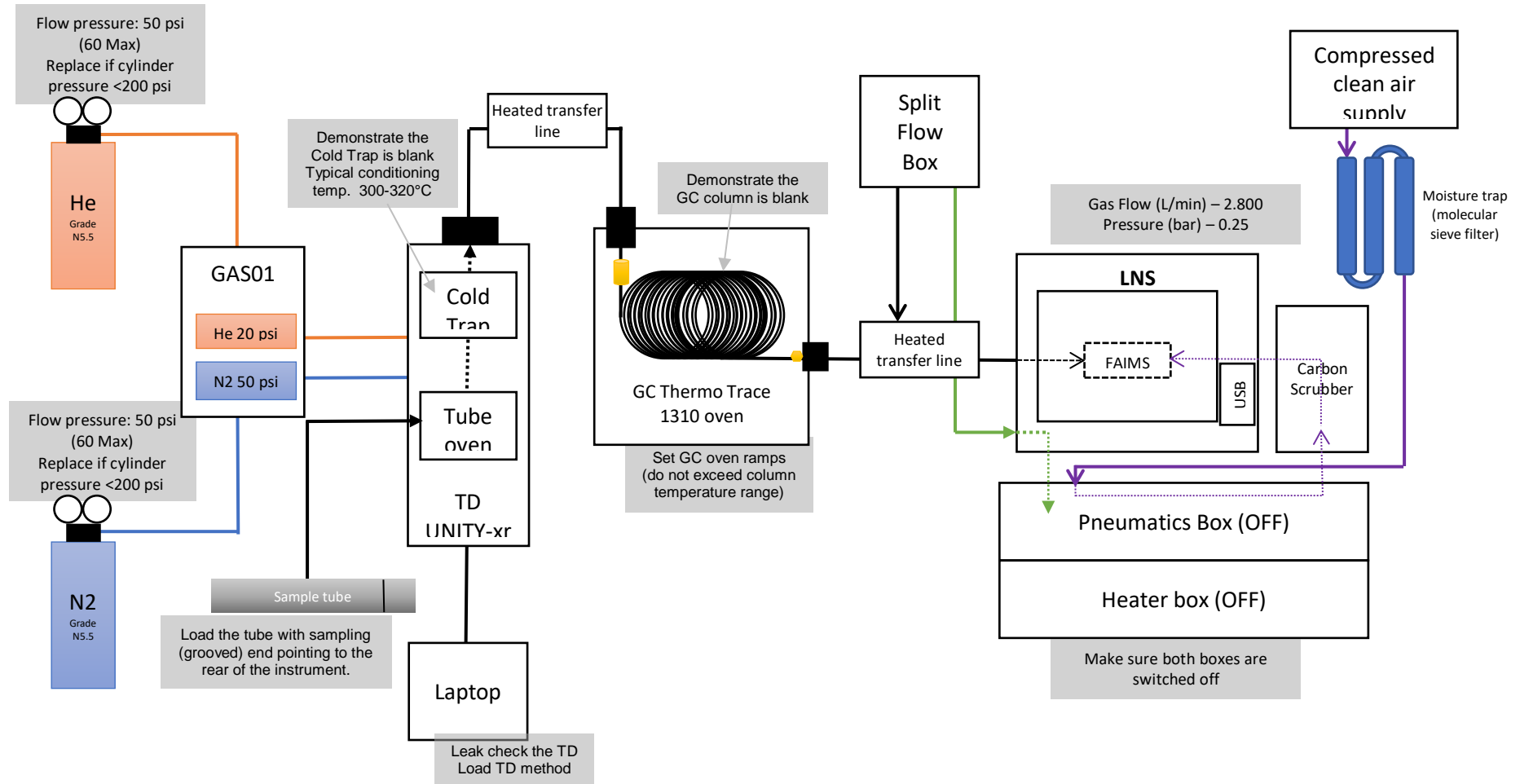


Figure 1. Schematic diagram of the TD-GC-LNS with the essential checks for sample running.

4 Procedure

4.1 TD-GC-LNS System Pre-Checks.

4.1.1 Ensure that the carrier (He) and purge (N2) gas cylinders are not empty and that the installation has been leak checked. Replace the cylinder if the pressure is less than 200PSI. Both gases should be 5.5 grade (i.e. 99.9995%). Check that the gas line toggle valves are opened. If a gas generator is feeding the system, ensure it is switched on and perform pertinent checks. The primary and secondary regulator pressures to be set are listed in Table 1

	Cylinder (primary) regulator	GAS01 (secondary) regulator
Carrier gas pressure	50psi	20psi
Purge gas pressure	50-60psi	50psi

Table 1. Gas line pressure settings.

4.1.2 Demonstrate the TD instrument is leak tight by running a leak test on the TD system.

4.1.3 Check the LNS and ensure the ATLAS pneumatic and heat boxes are on. The gas flow and pressure parameters, displayed at the top of the LNS interface, should be close to 2800mL/min and the pressure 0.25bar respectively.

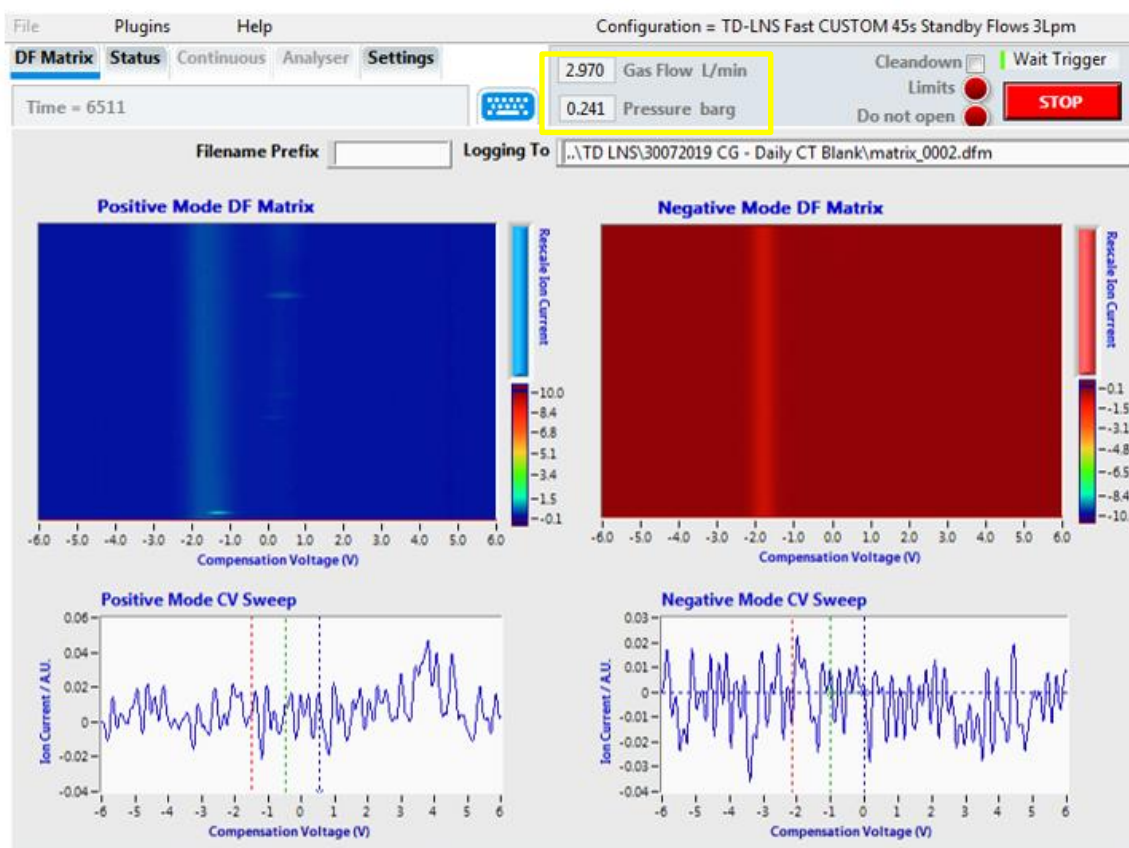


Figure 2. Location of the LNS gas flow and pressure parameters in the LNS interface.

- 4.1.4 If gas flow and pressure parameters are both zero, go to “Settings” and press the “COMM port” and “SFB settings” buttons, so that both display the message “Connect”, as shown in Figure 3.

Request technical support if the problem persists. For additional support, please visit www.owlstonemedical.com and click on [Support](#) or send an email to support@owlstone.co.uk.

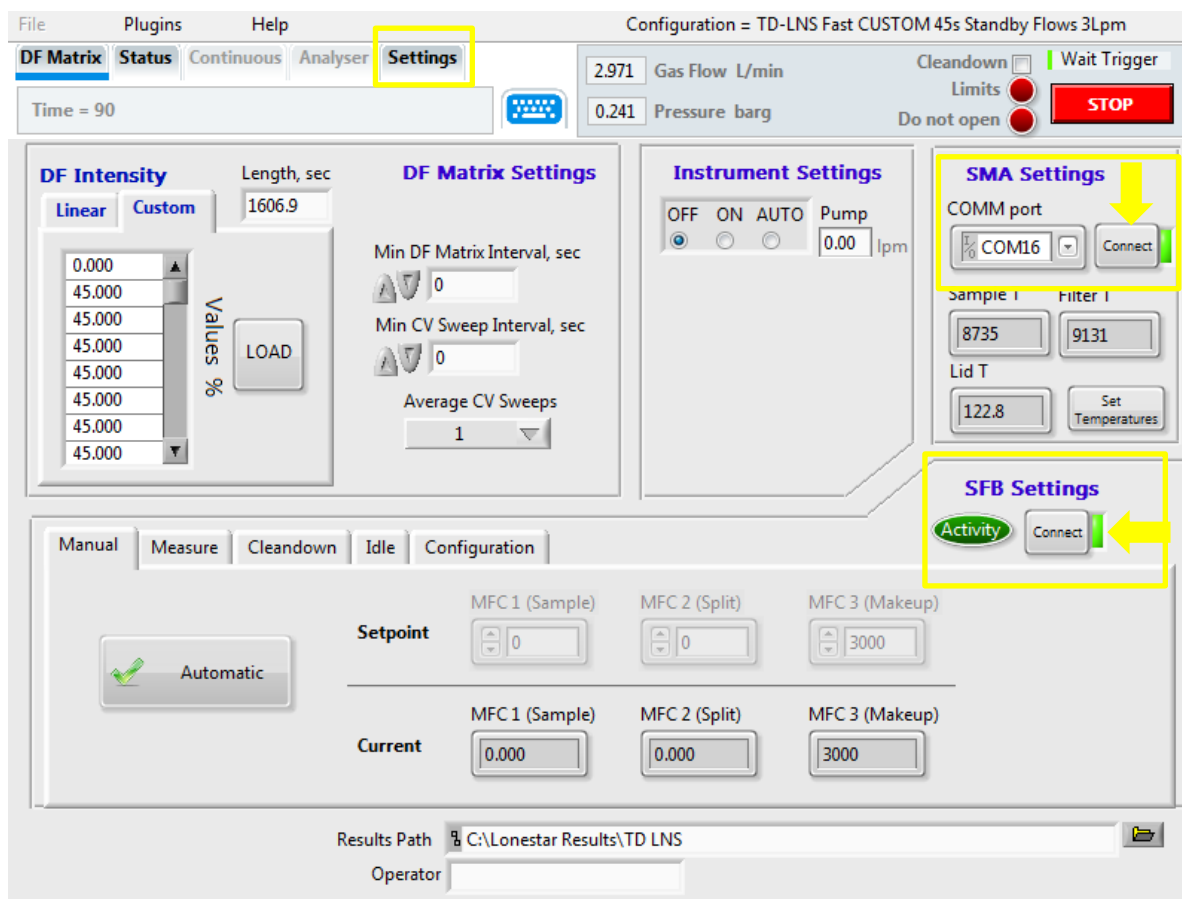


Figure 3. LNS interface screen. The yellow arrows point at the buttons that should be displaying “Connect”, press them if they are not.

- 4.1.5 Demonstrate the GC column is clean by running a column blank (see 95-0014 User Guide: TD-GC-Lonestar: How to run a column blank). Do not exceed the column temperature ranges.
- 4.1.6 Once the GC column has been proven to be clean, demonstrate the cold trap is clean by running a cold trap blank (see 95-0015 User Guide: TD-GC-Lonestar: How to run a Cold Trap Blank). The cold trap installed in the TD-GC-LNS system is the Markes U-T12ME-2S. The typical operating temperature ranges from 300-320°C, with a maximum operating temperature is 335 °C, which should never be exceeded.
- 4.1.7

4.2 GC Oven Ramp Rates Configuration.

Before running any sample, the GC oven ramp rates must be set. These must be defined, adapted and optimized by the user according to the analysis and the GC column. The GC oven ramp rates **must not** exceed the GC column temperature limit range. Column overheating leads to permanent

damage on the stationary phase, rendering the chromatographic separation ineffective. The temperature limit ranges for the HP-5 column (30m x 0.32 mm x 0.25µm, Agilent part number 19091J-413) is from -60°C to 325°C.

The GC oven ramp rates can be easily set by navigating in the GC interface. The Thermo Trace 1310 GC user interface is a touch screen. The main menu screen is shown in Figure 4.

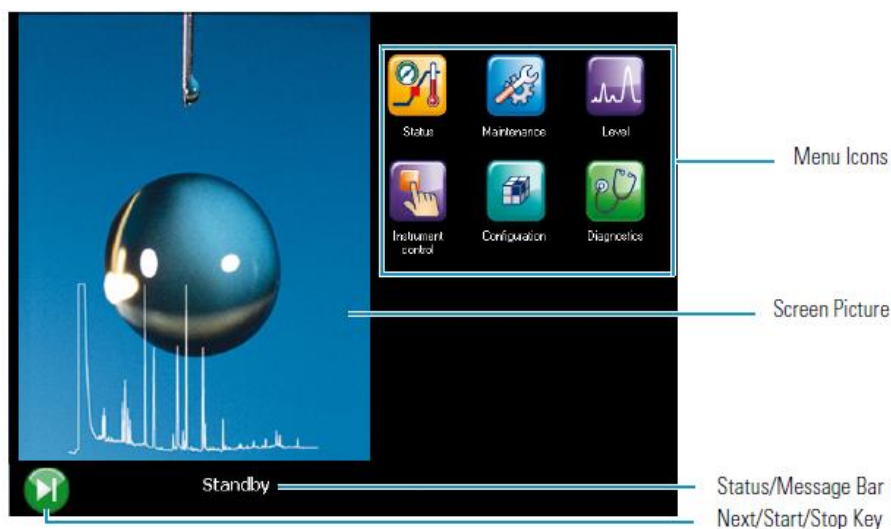


Figure 4. Thermo Trace GC Main menu interface.

4.2.1 From the main menu, tap in the “Instrument control” option (Figure 5). The instrument control menu will be displayed. Press the “Oven” icon, located at the top left corner, to open the submenu where the oven ramp rates configuration can be set.



Figure 5. Instrument control menu. Press “oven” in order to enter the GC oven ramp rates configuration.

4.2.2 The screen on Figure 6 will be displayed. Select the parameter to be adjusted by pressing on its value on the “Setpoint” column. Press “Clear” to delete the number, enter the correct value for each parameter and then “Enter” to save the changes (the changes will not apply otherwise). Press the home icon to return to the main menu showed in Figure 4.

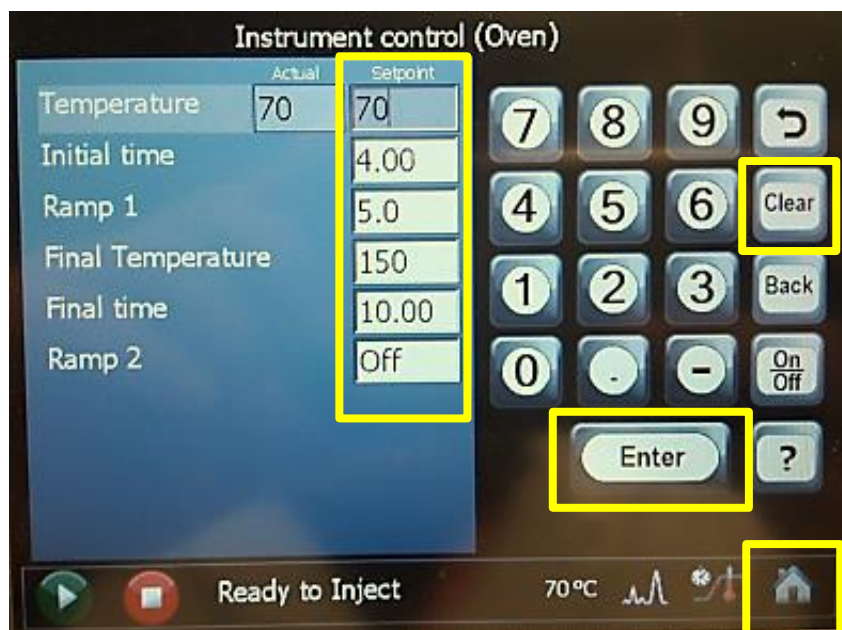


Figure 6. GC ramp rates configuration menu. Please, make sure to save the changes by pressing “Enter” and that the ramp rates do not exceed the GC column temperature limits.

4.2.3 From the main menu, press “Status” to monitor the status of the instrument. The popping up screen monitors the GC oven status during each stage in a run. This provides information about the current oven temperature, run progress, elapsed run time and remaining run time (these last two options are displayed during the run).



Figure 7. Status menu. Press on the “Oven temperature” to display the current temperature on the screen. In yellow, the current status of the GC. It requires to be “Ready to inject” in order to start the sample analysis.

4.2.4 The schematic to the bottom left corner of the screen (Figure 8) indicates the stage at which the GC oven is during each run. The GC is required to be at the “Ready to inject” stage in order to be recognized as “GC ready” by the Markes TD software (see Figure 16), and to start the run.

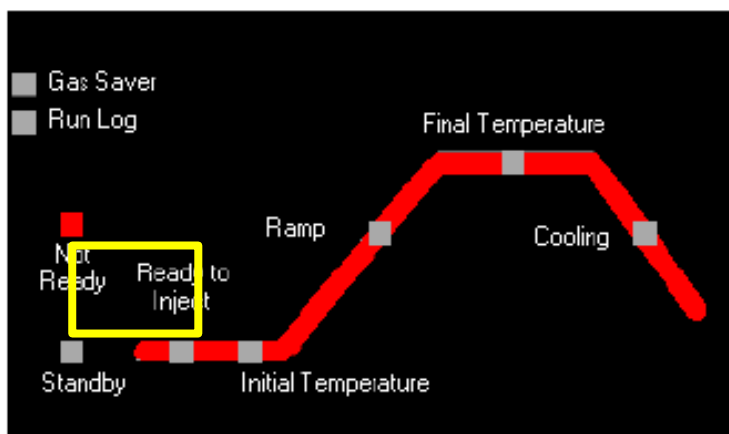


Figure 8. Run ramp stages schematic. “Not Ready” – The GC is waiting for parameters to become ready. This option is seen for example when the GC oven is reaching the temperature set. “Standby” – all parameters have reached the ready condition. “Ready to inject” – preparatory phase has finish and the analytical run can begin, the sample or autosampler can load the sample.

4.2.5 Once the oven has reached the temperature and conditions required for the run, the status bar to the bottom of the screen will display “Ready to Inject”, and a green start button appears on the right side of the panel (Figure 9). Do **not** press the “Start” button. The Thermo Trace 1310 is communicated with the UNITY-xr thermal desorption unit in such a way that the cold trap desorption triggers the start of the GC oven cycle.



Figure 9. Ready to inject. Do not press the start button for sample analysis. Start button is only used for GC column blanks.

4.2.6 The GC oven is ready. The ramp rates will be synchronized with the cold trap desorption.

4.3 Setting up the Lonestar For Data Collection.

4.3.1 Press the red “STOP” button, located to the upper right corner of the LNS interface (Figure 10).

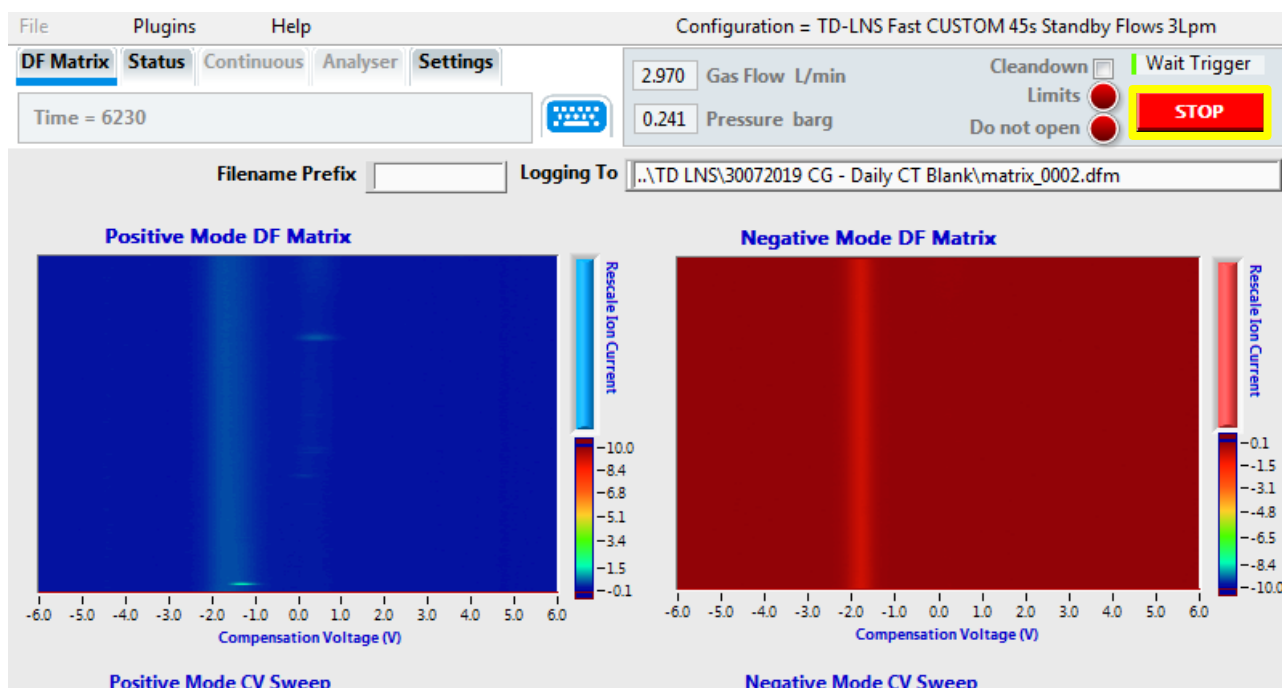


Figure 10. LNS Interface.

4.3.2 The red “STOP” button will be replaced by the green “START” button, and the LNS status will be “Idle”. Press the “START” button, enter the folder name in the “Select Logging Session” menu. Press “Log Data” to create the folder in the LNS directory in where DF matrices will be stored.

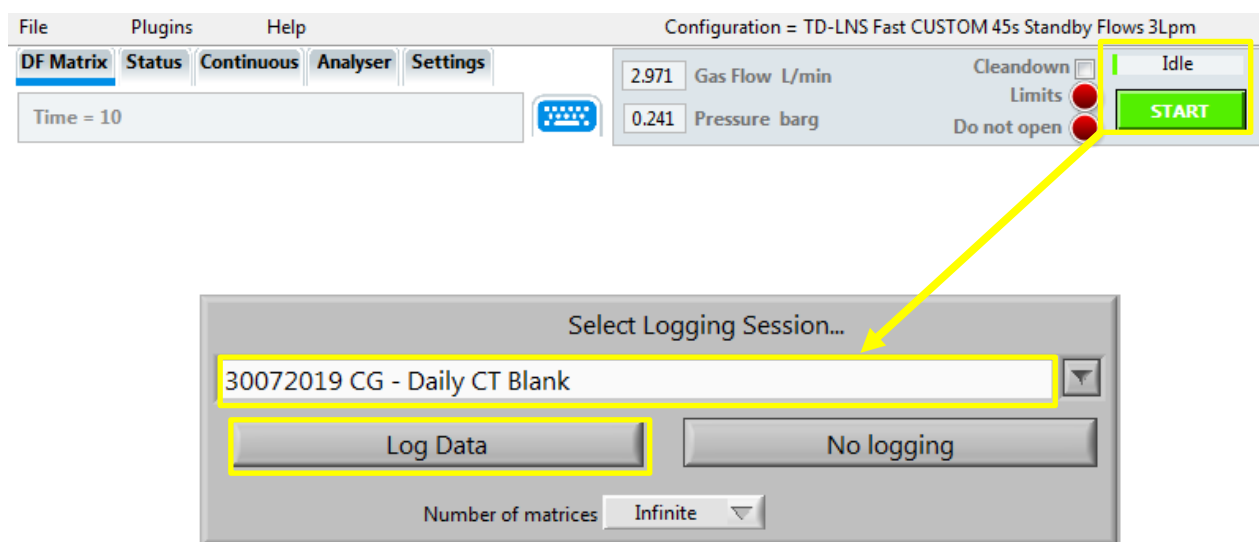


Figure 11. File name and log data option.

4.3.3 The DF matrices and the CV sweep graphs will then be empty. The LNS status will be on “Wait Trigger”. The “Logging To” will show the previous file save path, which will be updated once the analysis has finished.

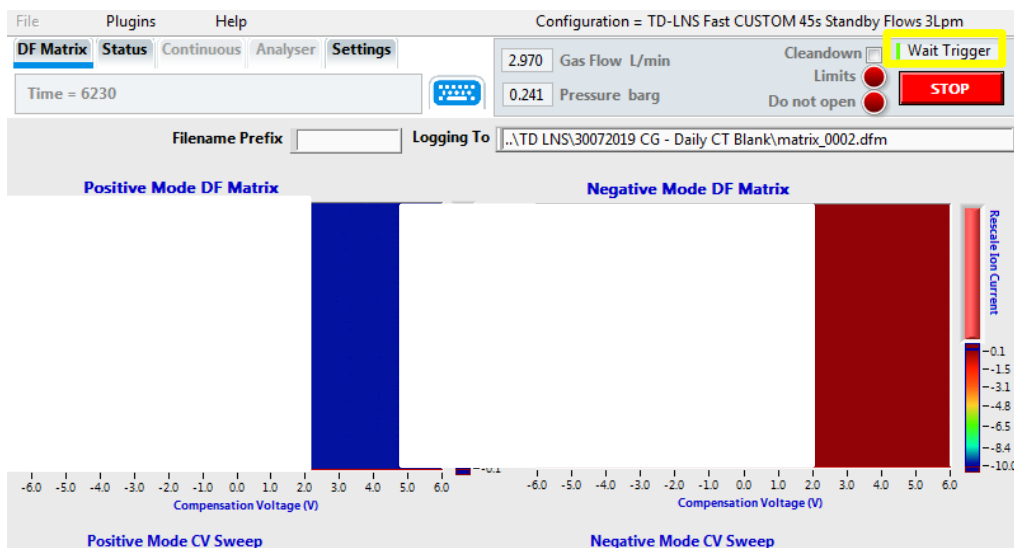


Figure 12. LNS ready for the analysis. Status must be on “Wait Trigger” and DF matrices will be generated as the sample is detected.

4.3.4 The LNS is ready to start the analysis. The LNS status will change to “Acquire Data” (Figure 13) after the cold trap desorption. DF matrices will be generated as the sample is run.

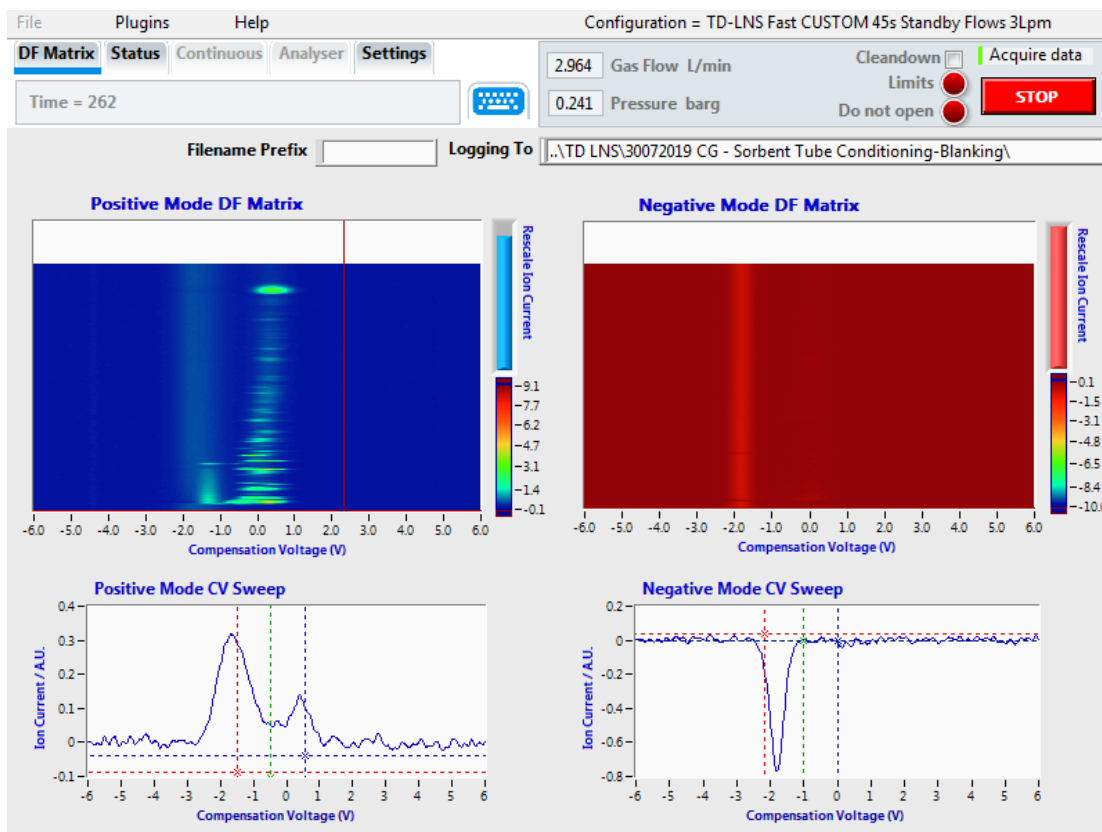


Figure 13. LNS interface during sample analysis. Notice that the LNS status is now “Acquire data” and that the DF matrices are generated as the analytes are detected.

4.3.5 The run will finish once the LNS has displayed a complete set of DF matrices on its interface. Data file can be found on the save path selected on 4.3.2.

4.4 Sample Tube Loading Into The UNITY-xr Tube Oven.

4.4.1 Sample tubes desorbed can be capped using DiffLok caps from Markes, which preserves the sample and tube integrity prior analysis. If an autosampler ULTRA-xr is attached, tubes capped with DiffLok caps can be loaded directly onto the autosampler plates.

4.4.2 To load tubes onto the UNITY-xr tube oven, use the left-hand lever mechanism. Before inserting a sample, ensure the tube oven is relatively cool and remove the DiffLok caps. Orientate the tube such that the sampling grooved end is pointing the rear of the instrument (Figure 14).

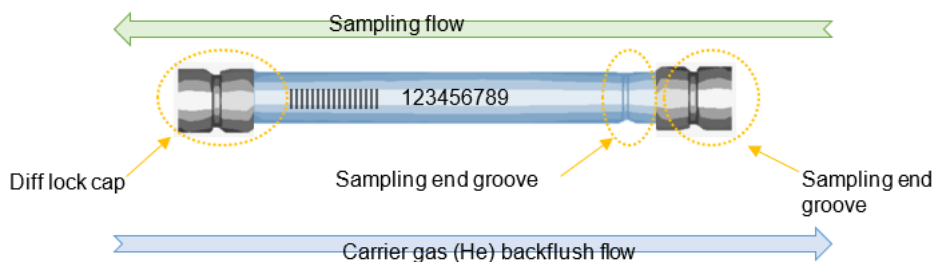


Figure 14. Sorbent tube diagram showing the sampling and carrier gas flows.

4.5 Setting Sequences in the Markes Instrument Controller (MIC).

4.5.1 Launch the MIC software by clicking on the desktop icon or selecting Start > Programs > Markes International > TD. The MIC homepage (Figure 15) will appear. Wait until the instrument status is "Idle" and then tap on "Sequence".

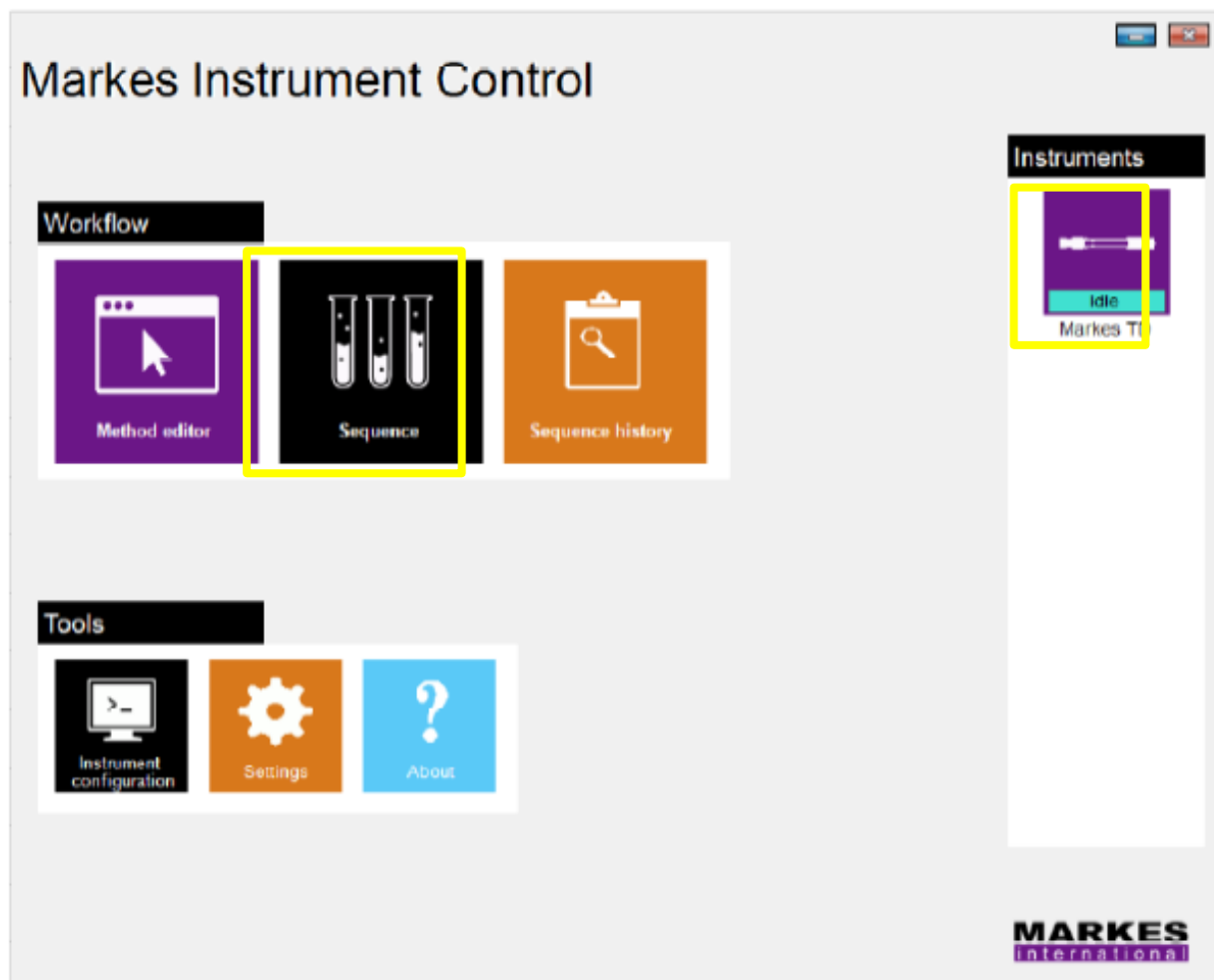


Figure 15. Markes Instrument Control (MIC) homepage.

4.5.2 Click on “Sequence” to open the “Sequence Edit View”. Sequences can be created and run from this menu. Click on the “Instruments” bar to display the schematic overview.

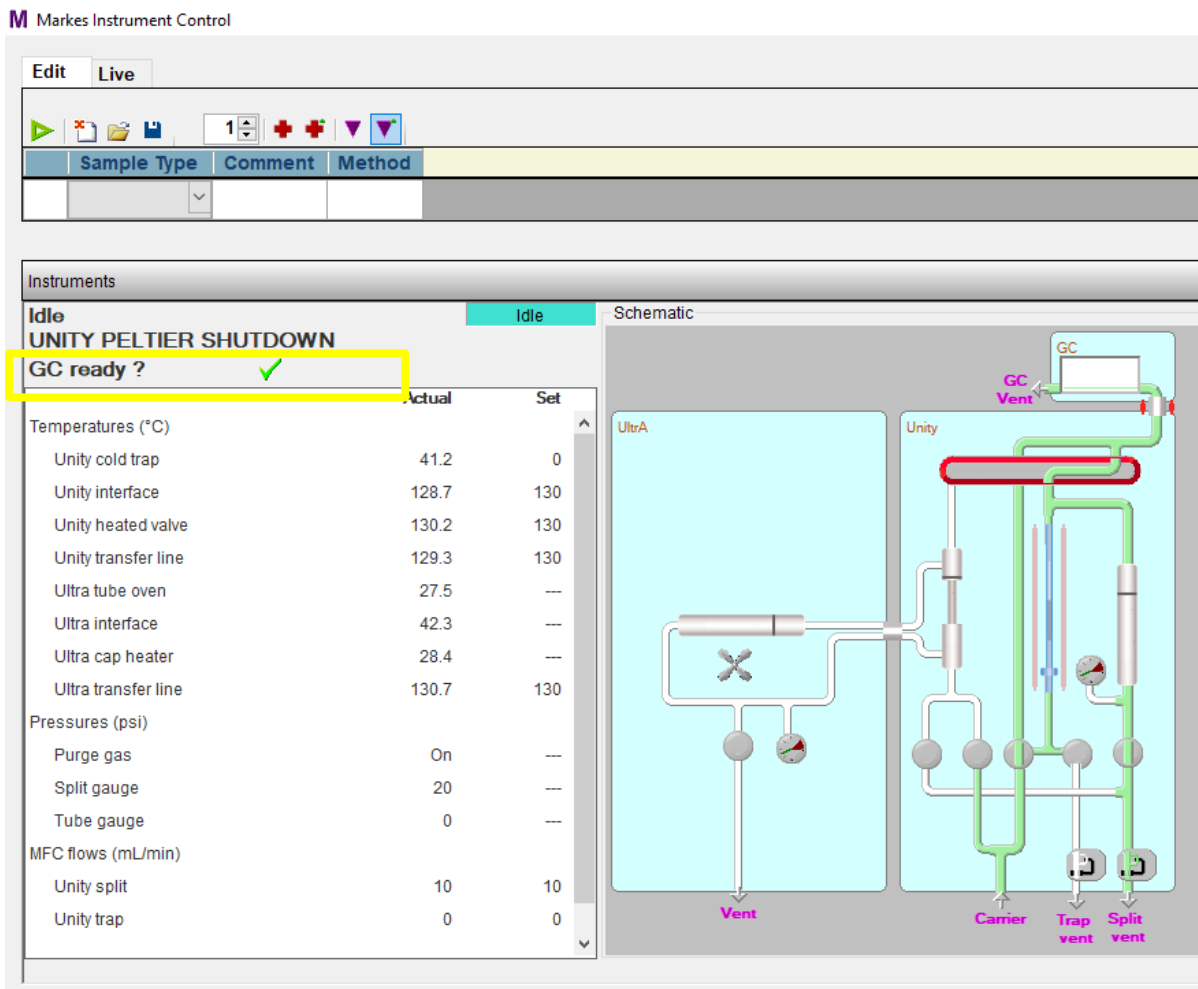


Figure 16. Sequence Edit View screen. The “GC ready?” is ticked as the GC oven has already been set.

4.5.3 Select “Sample” from the “Sample Type” scroll up menu (Figure 17).

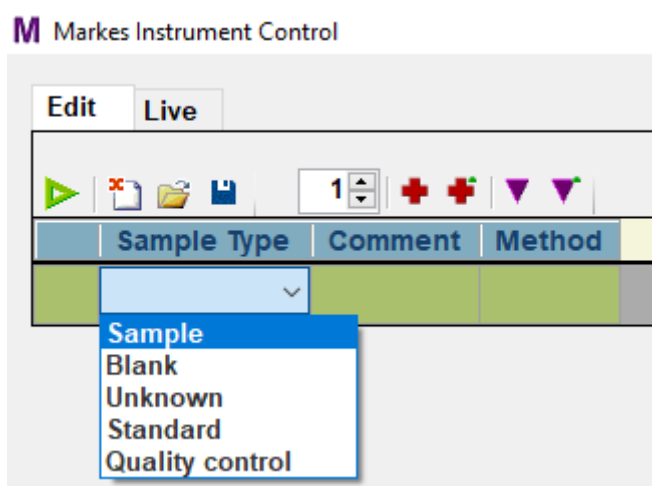


Figure 17. Sample Type selection menu.

4.5.4 Click on the “Method” box to open the methods list. Select the method to be loaded for the sample analysis. Once selected, the method will appear in the Sequence Edit View as per Figure 18.

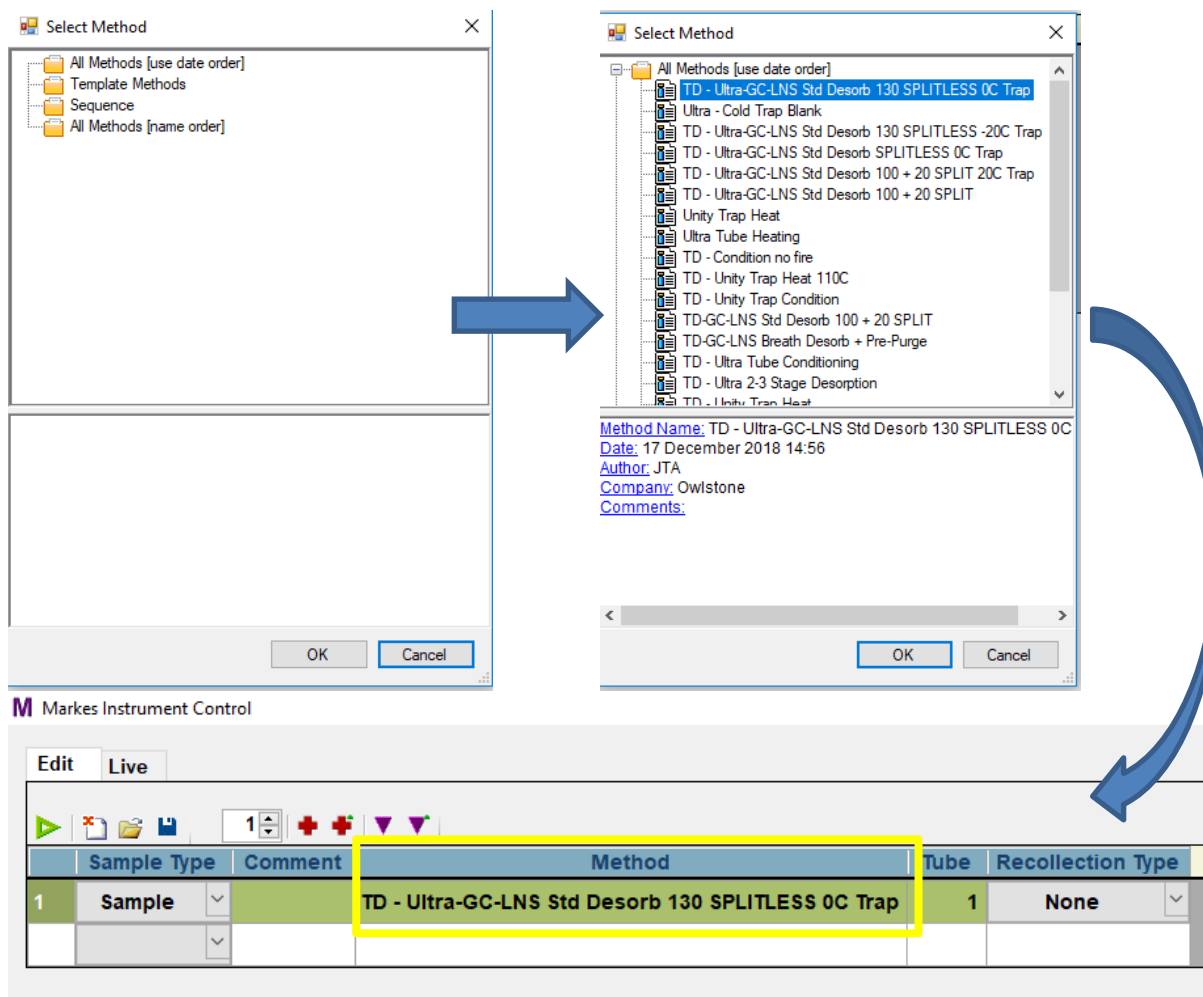


Figure 18. TD method selection.

- 4.5.5 Ensure that the correct GC oven ramp rates have been set in the GC oven, and that it is in the “Ready to inject” stage (section 4.2.5).
- 4.5.6 Check that the LNS is in the “Waiting for trigger” stage (section 4.3.3).
- 4.5.7 In the Sequence Edit View, at the top of the “Instrument” panel, check that the “GC ready?” shows a green tick and that the instrument is in the “Idle” stage.
- 4.5.8 Click on the green Start button at the top of the Sequence Edit View (Figure 19). The “Run Sequence” window will pop up. Make sure all options are unticked and press “OK”.

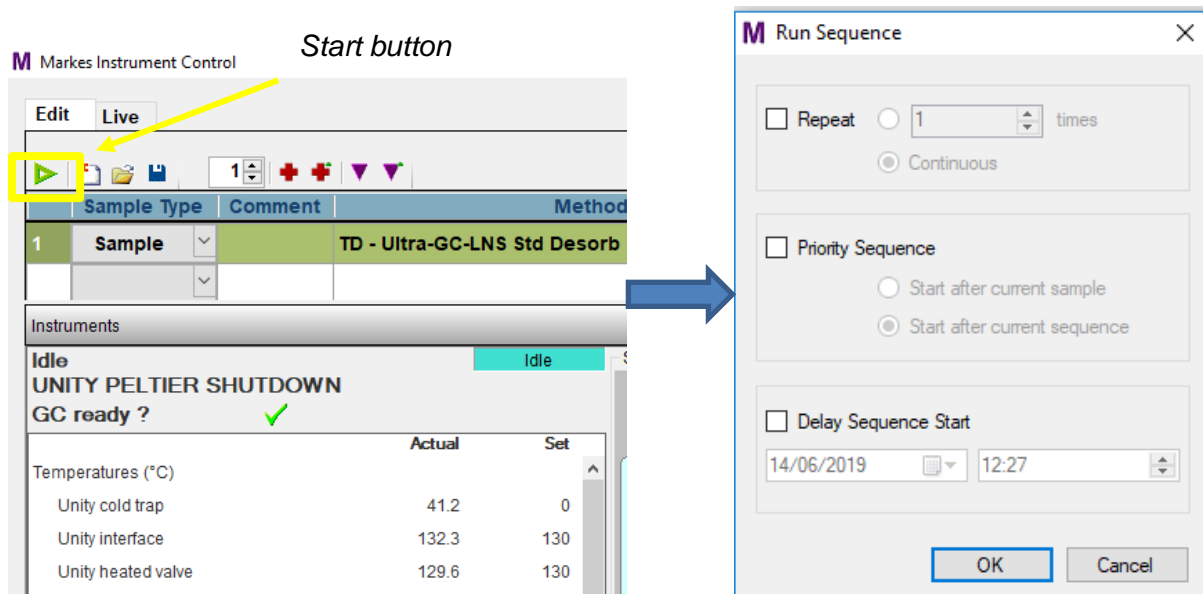


Figure 19. Press the Start button to begin the sequence run.

- 4.5.9 After pressing “OK”, the instrument will exit the “Idle” stage and will enter the “Active” stage, meaning the tube run has begun. The panel will show the current sequence stage as well as the elapsed time.



Figure 20. TD unit in the Active stage during the “Equilibrating” step.

- 4.5.10 When the TD reaches the “Trap Desorb” step, the GC and the LNS will be triggered, i.e., the oven ramp rate cycle will begin and the LNS will start detecting and collecting data.
- 4.5.11 The sample tube run ends once the LNS has finished generating DF matrix (see section 4.3.5). The data files can now be exported and processed.

5 Contacts and Support

The Owlstone Medical Ltd team is dedicated to providing excellent support. For all technical and safe use question relating to this manual, contact as at:

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Or email support at **support@owlstone.co.uk**